



PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁴ : A61K 37/02, C07K 7/08, 7/10	A1	(11) International Publication Number: WO 90/04408 (43) International Publication Date: 3 May 1990 (03.05.90)					
<p>(21) International Application Number: PCT/US89/04664</p> <p>(22) International Filing Date: 18 October 1989 (18.10.89)</p> <p>(30) Priority data:</p> <table border="0"> <tr> <td>261,237</td> <td>21 October 1988 (21.10.88)</td> <td>US</td> </tr> <tr> <td>353,618</td> <td>18 May 1989 (18.05.89)</td> <td>US</td> </tr> </table> <p>(71) Applicant: MAGAININ SCIENCES, INC. [US/US]; 550 Pinetown Road, Ft. Washington, PA 19034 (US).</p> <p>(72) Inventors: ZASLOFF, Michael ; 274 Linden Lane, Merion Station, PA 19066 (US). STEINBERG, Wallace, H. ; 76 Buena Vista Avenue, Rumson, NJ 07760 (US).</p> <p>(74) Agents: OLSTEIN, Elliot, M. et al. ; Carella, Byrne, Bain & Giffilian, 6 Becker Farm Road, Roseland, NJ 07068 (US).</p>	261,237	21 October 1988 (21.10.88)	US	353,618	18 May 1989 (18.05.89)	US	<p>(81) Designated States: AT (European patent), AU, BE (European patent), BG, BR, CH (European patent), DE (European patent), DK, FI, FR (European patent), GB (European patent), HU, IT (European patent), JP, KR, LU (European patent), NL (European patent), NO, RO, SE (European patent), SU.</p> <p>Published <i>With international search report.</i></p>
261,237	21 October 1988 (21.10.88)	US					
353,618	18 May 1989 (18.05.89)	US					
<p>(54) Title: COMPOSITION AND TREATMENT WITH BIOLOGICALLY ACTIVE PEPTIDES AND CERTAIN ANIONS</p> <p>(57) Abstract</p> <p>An amphiphilic ion-channel forming peptide and a toxic anion are employed as a pharmaceutical.</p>							

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	ES	Spain	MG	Madagascar
AU	Australia	FI	Finland	ML	Mali
BB	Barbados	FR	France	MR	Mauritania
BE	Belgium	GA	Gabon	MW	Malawi
BF	Burkina Faso	GB	United Kingdom	NL	Netherlands
BG	Bulgaria	HU	Hungary	NO	Norway
BJ	Benin	IT	Italy	RO	Romania
BR	Brazil	JP	Japan	SD	Sudan
CA	Canada	KP	Democratic People's Republic of Korea	SE	Sweden
CF	Central African Republic	KR	Republic of Korea	SN	Senegal
CG	Congo	LI	Liechtenstein	SU	Soviet Union
CH	Switzerland	LK	Sri Lanka	TD	Chad
CM	Cameroon	LU	Luxembourg	TG	Togo
DE	Germany, Federal Republic of	MC	Monaco	US	United States of America
DK	Denmark				

- 1 -

Composition and Treatment with Biologically Active Peptides and Certain Anions

This invention relates to biologically active peptides, and more particularly to compositions and uses involving biologically active peptides and certain anions.

In accordance with an aspect of the present invention, there is provided a composition which includes at least one biologically active amphiphilic peptide and/or biologically active protein; and a toxic anion.

In accordance with another aspect of the present invention, there is provided a process wherein there is administered to a host at least one biologically active amphiphilic peptide which is an ion channel-forming peptide and/or a biologically active protein which is an ion channel forming protein; and a toxic anion.

An ion channel-forming peptide or protein or ionophore is a peptide or protein which increases the permeability for ions across a natural or synthetic lipid membrane. B. Christensen et al. PNAS V 1. 85 P. 5072-76 (July, 1988) describes method 1 by which

-2-

indicates whether or not a peptide or protein has ion channel-forming properties and is therefore an ionophore. As used herein an ion channel-forming peptide or ion channel forming protein is a peptide or protein which has ion channel-forming properties as determined by the method of Christensen et al.

In accordance with yet another aspect of the present invention, there is provided a method of testing, or screening a sample for ion channel-forming peptides or proteins.

In accordance with this method, a first portion of an unknown sample is contacted with a sample containing target cells (e.g., bacterial cells, fungal cells, protozoa, tumor cells, virally infected cells, etc.), and the biological activity of the sample against the particular target cells is then measured. A second portion of the unknown sample to which a quantity of fluoride ion has been added is contacted with another sample containing target cells of the same type which were contacted by the first portion of unknown sample. The biological activity of the second portion of unknown sample and fluoride ion against the target cells is then measured. If the second portion of unknown sample and fluoride ion has greater biological activity against the target cells than does the first portion, to which no fluoride ion was added, then the unknown sample contains ion channel-forming peptides or proteins. In a preferred embodiment, the target cell is a bacterium.

An amphiphilic peptide is a peptide which includes both hydrophobic and hydrophilic peptide regions.

-3-

A toxic anion is one which when introduced into a target cell inhibits and/or prevents and/or destroys the growth of the target cell.

Such a toxic anion is one which in the absence of the ion channel forming peptide or protein is unable to cross a natural or synthetic lipid membrane; in particular a cell membrane, in sufficient amounts to adversely affect a cell.

In accordance with an aspect of the present invention wherein the biologically active peptide or protein and toxic anion are administered to a host, such biologically active peptide or protein and toxic anion may be administered as a single composition or in separate compositions, and the single or separate compositions may include additional materials, actives and/or inactives, in addition to the peptide or protein and/or toxic anion.

The ion channel-forming peptides employed in the present invention are generally water soluble to a concentration of at least 20 mg/ml at neutral pH in water. In addition, such peptides are non-hemolytic; i.e., they will not rupture blood cells at effective concentrations. In addition, the structure of such peptide provides for flexibility of the peptide molecule. When the peptide is placed in water, it does not assume an amphiphilic structure. When the peptide encounters an oily surface or membrane, the peptide chain folds upon itself into a rod-like structure.

In general, such peptides have at least 16 amino acids, and preferably at least 20 amino acids. In most cases, such peptides do not have in excess of 40 amino acids.

In general, such toxic anion is employed as part of a suitable compound. As representative examples

-4-

of such toxic anions, there may be mentioned: fluoride, peroxide and bicarbonate.

In employing both an ion channel-forming biologically active amphiphilic peptide or an ion channel forming protein, and a toxic anion, whether administered or prepared in a single composition, or in separate compositions, the peptide or protein and the toxic anion, are employed in amounts effective to inhibit and/or prevent and/or destroy the growth of the target cell. In effect, the anion potentiates the action of the peptide or protein. The term "potentiate," as employed herein, means that the amount of toxic anion is effective to reduce the minimum effective concentration of the peptide or protein for inhibiting growth of a target cell.

In general, the peptide or protein is employed to provide peptide dosages of from 1 mg to 500 mg per kilogram of host weight, when administered systemically. When administered topically, the peptide or protein is used in a concentration of from .05% to 5%.

The toxic anion, when used topically, is generally employed in a concentration of from 0.05% to 2.0%. When used systemically, the anion is generally employed in an amount of from 1 to 10 mg per kg of host weight.

The use of a combination of peptide or protein and toxic anion in accordance with the present invention is effective as an antibiotic, and may be employed to inhibit, prevent or destroy the growth or proliferation of microbes, such as bacteria and fungi. Similarly, such compositions may be employed as an anti-viral composition to inhibit, prevent or destroy the growth or proliferation of viruses.

-5-

Such compositions may also be employed as a spermicide to inhibit, prevent or destroy the motility of sperm.

Such compositions may also be employed as anti-tumor agents to inhibit the growth of or destroy tumors.

Such compositions may also be used as anti-parasitic agents to inhibit the growth of or destroy parasites.

The compositions have a broad range of potent antibiotic activity against a plurality of microorganisms, including gram-positive and gram-negative bacteria, fungi, protozoa and the like. Such compositions may be employed for treating or controlling microbial infection caused by organisms which are sensitive to such composition. The treatment may comprise administering to a host organism or tissues acceptable to or affiliated with a microbial infection an anti-microbial amount of peptide or protein and toxic anion.

The compositions may also be used as preservatives or sterilants for materials susceptible to microbial contamination.

In accordance with a preferred embodiment, the peptide used in conjunction with a toxic anion is a basic (positively charged) polypeptide having at least sixteen amino acids wherein the polypeptide includes at least eight hydrophobic amino acids and at least eight hydrophilic amino acids. Still more particularly, the hydrophobic amino acids are in groups of two adjacent amino acids, and each group of two hydrophobic amino acids is spaced from another group of two hydrophobic amino acids by at least one amino acid other than a hydrophobic amino acid (preferably at least two amino acids) and generally

-6-

by no greater than four amino acids, and the amino acids between pairs of hydrophobic amino acids may or may not be hydrophilic.

The hydrophilic amino acids are generally also in groups of two adjacent amino acids in which at least one of the two amino acids is a basic hydrophilic amino acid, with such groups of two hydrophilic amino acids being spaced from each other by at least one amino acid other than a hydrophilic amino acid (preferably at least two amino acids) and generally no greater than four amino acids, and the amino acids between pairs of hydrophilic amino acids may or may not be hydrophobic.

In accordance with a particularly preferred embodiment, the polypeptide comprises a chain of at least four groups of amino acids, with each group consisting of four amino acids. Two of the four amino acids in each group are hydrophobic amino acids, and two of the four amino acids in each group are hydrophilic, with at least one of the hydrophilic amino acids in each group being a basic hydrophilic amino acid and the other being a basic or neutral hydrophilic amino acid.

The hydrophobic amino acids may be selected from the class consisting of Ala, Cys, Phe, Gly, Ile, Leu, Met, Val, Trp, and Tyr. The neutral hydrophilic amino acids may be selected from the class consisting of Asn, Gln, Ser, and Thr. The basic hydrophilic amino acids may be selected from the class consisting of Lys, Arg, His and ornithine (O).

Each of the groups of four amino acids may be of the sequence ABCD, BCDA, CDAB, or DABC, wherein A and B are each hydrophobic amino acids and may be the same or different, and C or D is a basic hydrophilic amino acid, and the other of C or D is a

-7-

basic or neutral hydrophilic amino acid and may be the same or different. In a preferred embodiment, the polypeptide chain may comprise 5 or 6 groups of this sequence. In each group, each of A, B, C and D may be the same in some or all of the groups or may be different in some or all of the groups.

The polypeptide chain preferably has at least 20 amino acids, and no greater than 50 amino acids. It is to be understood, however, that the polypeptide does not have to consist entirely of the groups described above. The polypeptide may have amino acids extending from either or both ends of the noted groups forming the polypeptide chain and/or there may be amino acids between one or more of the at least four groups and still remain within the scope of the invention.

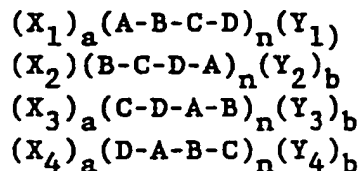
The groups of amino acids may be repeating groups of amino acids, or the amino acids in the various groups may vary provided that in each group of the at least four groups of amino acids there are two hydrophobic and two hydrophilic amino acids as hereinabove noted.

Thus, in a preferred embodiment, the biologically active polypeptide comprises a chain including at least four groups of amino acids, each containing four amino acids. Two of the four amino acids in each group are hydrophobic, at least one amino acid is basic hydrophilic, and the remaining one is basic or neutral hydrophilic, with the polypeptide chain preferably having at least 20 amino acids but no greater than 50 amino acids.

In one embodiment, each of the at least four groups of amino acids which are in the peptide chain is of the sequence A-B-C-D, B-C-D-A, C-D-A-B or D-A-B-C wherein A and B are hydrophobic amino acids,

-8-

one of C or D is basic hydrophilic amino acid, and the other of C or D is basic or neutral hydrophilic amino acid. The resulting polypeptide chain, therefore, may have one of the following sequences:



wherein X_1 is D; C-D- or B-C-D-, Y_1 is -A or -A-B or -A-B-C

X_2 is A-, D-A- or C-D-A-

Y_2 is -B, -B-C or B-C-D

X_3 is B-, A-B-, D-A-B-

Y_3 is -C, -C-D, -C-D-A

X_4 is C-, B-C-, A-B-C-

Y_4 is -D, -D-A, -D-A-B

a is 0 or 1; b is 0 or 1

and n is at least 4

It is to be understood that the peptide chain may include amino acids between the hereinabove noted groups of four amino acids provided that the spacing between such groups and the charge on the amino acids does not change the characteristics of the peptide chain which provide amphiphilicity and a positive charge and do not adversely affect the folding characteristics of the chain to that which is significantly different from one in which the hereinabove noted group of four amino acids are not spaced from each other.

As representative examples of peptides in accordance with the present invention, there may be mentioned.

I Ala-Phe-Ser-Lys-Ala-Phe-Ser-Lys-Ala-Phe-Ser-

-9-

Lys-Ala-Phe-Ser-Lys-Ala-Phe-Ser-Lys

II Ala-Phe-Ser-Lys-Ala-Phe-Ser-Lys-Ala-Phe-Ser-Lys-Ala-Phe-Ser-Lys-Ala-Phe-Ser-Lys.

III Phe-Ser-Lys-Ala-Phe-Ser-
Lys-Ala-Phe-Ser-Lys-Ala-
Phe-Ser-Lys-Ala-

IV Ser-Lys-Ala-Phe-Ser-Lys-Ala-
Phe-Ser-Lys-Ala-Phe-Ser-Lys-Ala-
Phe-Ser-Lys-Ala-Phe-

V Lys-Ala-Phe-Ser-Lys-Ala-Phe-Ser-Lys-Ala-Phe-Ser-
Lys-Ala-Phe-Ser

The peptide, may have amino acids extending from either end of the chain. For example, the chains may have a Ser-Lys sequence before the "Ala" end, and/or an Ala-Phe sequence after the "Lys" end. Other amino acid sequences may also be attached to the "Ala" and/or the "lys" end.

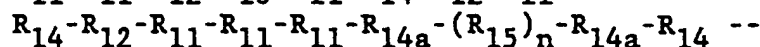
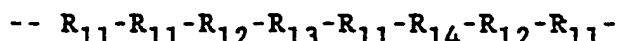
Similarly, in any polypeptide chain having at least four groups of amino acids of the sequence as described above, the chain may have, for example, a C-D sequence before the first A-B-C-D group. Also other amino acid sequences may be attached to the "A" and/or the "D" end of one of these polypeptide chains. Also there may be amino acids in the chain which space one or more groups of the hereinabove noted four amino acids from each other.

The peptides may be produced by known techniques and obtained in substantially pure form. For example, the peptides may be synthesized on an automatic synthesizer. Journal of American Chemical Society, Vol 85 Pages 2149-54(1963). It is also possible to produce such peptides by genetic engineering techniques.

-10-

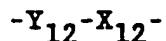
In accordance with another preferred embodiment, the peptide employed in conjunction with a toxic anion may be a magainin peptide.

A magainin peptide is either a magainin such as magainin I, II or III or an analogue or derivative thereof. The magainin peptides preferably include the following basic peptide structure X_{12}

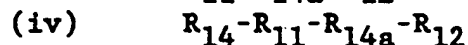
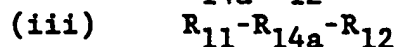
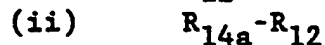


wherein R_{11} is a hydrophobic amino acid, R_{12} is a basic hydrophilic amino acid; R_{13} is a hydrophobic, neutral hydrophilic, or basic hydrophilic amino acid; R_{14} and R_{14a} are hydrophobic or basic hydrophilic amino acids; R_{15} is glutamic acid or aspartic acid, or a hydrophobic or a basic hydrophilic amino acid, and n is 0 or 1. In a preferred embodiment, R_{13} is a hydrophobic or neutral hydrophilic amino acid, R_{14a} is a hydrophobic amino acid, and R_{15} is glutamic acid or aspartic acid.

Thus, for example, a magainin peptide may include the following structure:

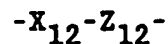


where X_{12} is the hereinabove described basic peptide structure and Y_{12} is



where R_{11} , R_{12} , R_{14} and R_{14a} are as previously defined.

A magainin peptide may also have the following structure:



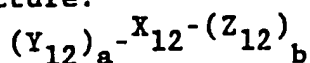
wherein X_{12} is as previously defined and Z_{12} is:

-11-

(i) R_{16} where R_{16} is a basic hydrophilic amino acid or asparagine or glutamine.

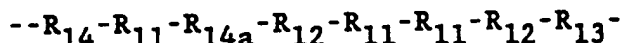
(ii) $R_{16}-R_{17}$ where R_{17} is a neutral hydrophilic amino acid, a hydrophobic amino acid, or a basic hydrophilic amino acid. Preferably, R_{17} is a neutral hydrophilic amino acid.

A magainin peptide may also have the following structure:



where X_{12} , Y_{12} and Z_{12} are as previously defined and a is 0 or 1 and b is 0 or 1.

The magainin peptides may also include the following basic peptide structure X_{13} :



$R_{11}-R_{14}-R_{12}-R_{11}-R_{11}-R_{12}-$, wherein R_{11} , R_{12} , R_{13} , R_{14} , and R_{14a} are amino acids as hereinabove described.

The magainin peptide may also include the following structure $X_{13}-Z_{13}$; wherein X_{13} is the hereinabove described basic peptide structure and Z_{13} is

$(R_{11})_n - (R_{11})_n - (R_{11})_n - (R_{14a})_n - (R_{15})_n - (R_{14a})_n - (R_{14})_n - (R_{16})_n - (R_{17})_n$ wherein R_{11} , R_{14} , R_{14a} , R_{15} , R_{16} , and R_{17} are as hereinabove described, and n is 0 or 1, and each n may be the same or different.

The magainin peptides generally include at least fourteen amino acids and may include up to forty amino acids. A magainin peptide preferably has 22 or 23 amino acids. Accordingly, the hereinabove described basic peptide structures of a magainin peptide may include additional amino acids at the amino end or at the carboxyl end, or at both ends.

As representative examples of such magainin peptides, there may be mentioned peptides having the following primary sequence (expressed as a single

-12-

letter code) as well as appropriate analogues and derivatives thereof:

- (a) (NH_2) GIGKFLHSAGKFGKAFVGEIMKS(OH) or (NH_2)
(Magainin I)
- (b) (NH_2) GIGKFLHSAKKFGKAFVGEIMNS(OH) or (NH_2)
(Magainin II)
- (c) (NH_2) GIGKFLHSAKKFGKAFVGEIMN(OH) or (NH_2)
(Magainin III)

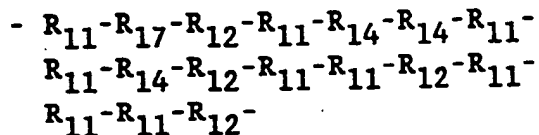
The following are examples of peptide derivatives or analogs of the basic structure:

- (d) (NH_2) IGKFLHSAKKFGKAFVGEIMNS(OH) or (NH_2)
- (e) (NH_2) GKFLHSAKKFGKAFVGEIMNS(OH) or (NH_2)
- (f) (NH_2) KFLHSAKKFGKAFVGEIMNS(OH) or (NH_2)

Magainin peptides are described in Proc. Natl. Acad. Sci. Vol. 84 pp. 5449-53 (Aug. 87). The term "magainin peptides" as used herein refers to the basic magainin structure as well as derivatives and analogs thereof, including but not limited to the representative derivatives or analogs.

In accordance with a further embodiment, the peptide employed in conjunction with a toxic anion may be a PGLa peptide or an XPF peptide.

A PGLa peptide is either PGLa or an analogue or derivative thereof. The PGLa peptides preferably include the following basic peptide structure X_{14} :



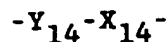
where R_{11} , R_{12} , R_{14} , and R_{17} are as previously defined.

The PGLa peptides generally include at least seventeen amino acids and may include as many as forty amino acids. Accordingly, the hereinabove described basic peptide structure for a PGLa peptide may include additional amino acids at the amino end

-13-

or at the carboxyl end or at both the amino and carboxyl end.

Thus, for example, a PGLa peptide may have the following structure:



where X_{14} is as previously defined and

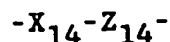
Y_{14} is

(i) R_{11} ;

(ii) $R_{14}-R_{11}$

where R_{11} and R_{14} are as previously defined.

For example, a PGLa peptide may also have the following structure:



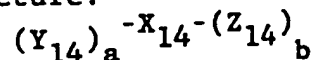
where X_{14} is as previously defined; and Z_{14} is:

(i) R_{11} ; or

(ii) $R_{11}-R_{11}$

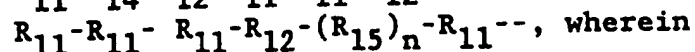
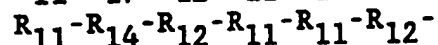
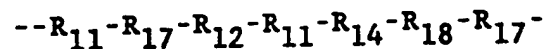
where R_{11} is as previously defined.

A PGLa peptide may also have the following structure:



where X_{14} ; Y_{14} and Z_{14} are as previously defined, a is 0 or 1 and b is 0 or 1.

An XPF peptide is either XPF or an analogue or derivative thereof. The XPF peptides preferably include the following basic peptide structure X_{16} :



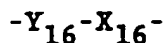
R_{11} , R_{12} , R_{14} , R_{15} and R_{17} are as previously defined and R_{18} is glutamine or asparagine or a basic hydrophilic, or hydrophobic amino acid and, n is 0 or 1.

The XPF peptides generally include at least nineteen amino acids and may include up to forty amino acids. Accordingly, the hereinabove described

-14-

basic peptide structure of XPF may include additional amino acids at the amino end, or at the carboxyl end or at both the amino and carboxyl ends.

Thus, for example, an XPF peptide may include the following structure:



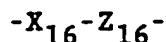
where X_{16} is as previously defined and Y_{16} is

(i) R_{11} or

(ii) $R_{14}-R_{11}$

where R_{11} and R_{14} are as previously defined.

An XPF peptide may include the following structure:



where X_{16} is as previously defined and Z_{16} is

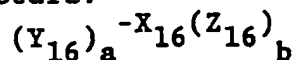
(i) R_{11} ; or

(ii) $R_{11}-R_{18}$; or

(iii) $R_{11}-R_{18}$ -Proline; or

(iv) $R_{11}-R_{18}$ -Proline- R_{12}

An XPF peptide may also have the following structure:



where X_{16} , Y_{16} and Z_{16} are as previously defined: a is 0 or 1 and b is 0 or 1.

Preferred are XPF or PGLa peptides, which are characterized by the following primary amino acid sequence(single letter amino acid code):

PGLa : GMASKAGAIAGKIAKVALKAL (NH_2)

XPF : GWASKIGQTLGKIAKVGLKELIQPK

A review of XPF and PGLa can be found in Hoffman et al, EMBO J. 2:711-714, 1983; Andreu et al, J. Biochem. 149:531-535, 1985; Gibson et al J. Biol. Chem. 261:5341-5349, 1986; and Giovannini et al, Biochem J. 243:113-120, 1987.

In accordance with yet another embodiment, the peptide employed in conjunction with a toxic anion

-15-

may be a CPF peptide or appropriate analogue or derivative thereof.

CPF peptides as well as analogues and derivatives thereof are herein sometimes referred to collectively as CPF peptides.

The CPF peptide is preferably one which includes the following peptide structure X_{30} :

$-R_{21}-R_{21}-R_{22}-R_{22}-R_{21}-R_{21}-R_{23}-R_{21}-$

$-R_{21}-R_{21}-R_{23}-R_{21}-R_{21}-R_{24}-R_{25}-R_{21}-$

wherein R_{21} is a hydrophobic amino acid;

R_{22} is a hydrophobic amino acid or a basic hydrophilic amino acid;

R_{23} is a basic hydrophilic amino acid; and

R_{24} is a hydrophobic or neutral hydrophilic amino acid; and

R_{25} is a basic or neutral hydrophilic amino acid.

The hereinabove basic structure is hereinafter symbolically indicated as X_{30} .

The hydrophobic amino acids are Ala, Cys, Phe, Gly, Ile, Leu, Met, Val, Trp, and Tyr.

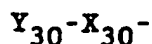
The neutral hydrophilic amino acids are Asn, Gln, Ser, and Thr.

The basic hydrophilic amino acids are Lys, Arg, His and ornithine.

The CPF peptide may include only the hereinabove noted amino acids or may include additional amino acids at the amino end or carboxyl end or both the amino and carboxyl end. In general, the peptide does not include more than 40 amino acids.

The CPF peptides including the above basic peptide structure may have from 1 to 4 additional amino acids at the amino end. Accordingly, such preferred peptides may be represented by the structural formula:

-16-



wherein X_{30} is the hereinabove described basic peptide structure and Y_{30} is

- (i) $R_{25}-$, or
- (ii) $R_{22}-R_{25}$; or
- (iii) $R_{21}-R_{22}-R_{25}$; or
- (iv) $R_{22}-R_{21}-R_{22}-R_{25}$; preferably
Glycine $-R_{21}-R_{22}-R_{25}-$

wherein R_{21} , R_{22} , and R_{25} are as previously defined.

The carboxyl end of the basic peptide structure may also have additional amino acids which may range from 1 to 13 additional amino acids.

In a preferred embodiment, the basic structure may have from 1 to 7 additional amino acids at the carboxyl end, which may be represented as follows:

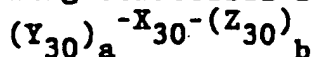
$-X_{30}-Z_{30}$ wherein

X_{30} is the hereinabove defined basic peptide structure and Z_{30} is

- (i) $R_{21}-$,
- (ii) $R_{21}-R_{21}-$;
- (iii) $R_{21}-R_{21}-R_{24}$;
- (iv) $R_{21}-R_{21}-R_{24}-R_{24}$;
- (v) $R_{21}-R_{21}-R_{24}-R_{24}-R_{26}$;
- (vi) $R_{21}-R_{21}-R_{24}-R_{24}-R_{26}-\text{Gln}$; or
- (vii) $R_{21}-R_{21}-R_{24}-R_{24}-R_{26}-\text{Gln}-\text{Gln}$,

wherein R_{21} and R_{24} are as previously defined, and R_{26} is proline or a hydrophobic amino acid.

Preferred peptides may be represented by the following structural formula:



wherein X_{30} , Y_{30} and Z_{30} are as previously defined and a is 0 or 1 and b is 0 or 1.

-17-

Representative examples of CPF peptides which are useful in the present invention some of which have been described in the literature and comprise the following sequences (single letter amino acid code):

- (1) GFGSFLGLALKALKIGANALGGAPQQ
- (2) GLASFLGKALKAGLKIGAHLLGGAPQQ
- (3) GLASLLGKALKAGLKIGTHFLGGAPQQ
- (4) GLASLLGKALKATLKIGTHFLGGAPQQ
- (5) GFASFLGKALKALKIGANMLGGTPQQ
- (6) GFGSFLGKALKALKIGANALGGAPQQ
- (7) GFGSFLGKALKALKIGANALGGSPQQ
- (8) GFASFLGKALKALKIGANLLGGTPQQ

A review of the CPF peptides can be found in Richter, K., Egger, R., and Kreil (1986) J. Biol. Chem. 261, 3676-3680; Wakabayashi, T. Kato, H., and Tachibana, S. (1985) Nucleic Acids Research 13, 1817-1828; Gibson, B.W., Poulter, L., Williams, D.H., and Maggio, J.E. (1986) J. Biol. Chem. 261, 5341-5349.

CPF peptides which may be employed in the present invention are represented by the following (single letter amino acid code):

G12S3LG4ALKA5LKIG678LGG9(10)QQ

Where:

- | | | |
|----|---|------------|
| 1 | = | F, L |
| 2 | = | G, A |
| 3 | = | F, L |
| 4 | = | K, L |
| 5 | = | A, G, T |
| 6 | = | A, T |
| 7 | = | H, N |
| 8 | = | A, M, F, L |
| 9 | = | A, S, T |
| 10 | = | P, L |

-18-

The numbered amino acids may be employed as described in any combination to provide either a basic CPF peptide structure or an analogue or derivative. The term CPF peptide includes the basic peptide structure as well as analogs or derivatives thereof.

In still another embodiment, the peptide employed in conjunction with a toxic anion is a cecropin. The cecropins and analogs and derivatives thereof are described in Ann. Rev. Microbiol 1987 Vol. 41 pages 103-26, in particular p. 108 and Christensen et al PNAS Vol. 85 p. 5072-76, which are hereby incorporated by reference.

The term cecropins includes the basic structure as well as analogues and derivatives.

In yet another embodiment, the peptide employed in conjunction with the toxic anion is a sarcotoxin. The sarcotoxins and analogs and derivatives thereof are described in Molecular Entomology pages 369-78 in particular p. 375 Alan R. Liss Inc. (1987), which is hereby incorporated by reference.

The term sarcotoxin includes the basic materials as well as analogues and derivatives.

In another embodiment, an ion channel-forming protein may be used in conjunction with a toxic anion. Ion channel-forming proteins which may be employed include defensins, also known as human neutrophil antimicrobial peptides (HNP), major basic protein (MBP) of eosinophils, bactericidal permeability-increasing protein (BPI), and a pore-forming cytotoxin called variously perforin, cytolysin, or pore-forming protein. Defensins are described in Selsted, et al., J. Clin. Invest., V 1. 76, pgs. 1436-1439 (1985). MBP proteins are described in Wasman, et al., J. Biol. Chem., Vol.

-19-

263, pgs 12559-12563. (1988). BPI proteins are described in Ooi, et al, J. Biol. Chem., Vol. 262, pgs. 14891-14894 (1987). Perforin is described in Henkart, et al., J. Exp. Med., 160: 75 (1984), and in Podack, et al., J. Exp. Med., 160:695 (1984). The above articles are hereby incorporated by reference.

The term ion channel-forming proteins includes the basic structures of the ion-forming proteins as well as analogues and derivatives.

The present invention will be further described with respect to the following examples, however, the scope of the invention is not to be limited thereby.

TABLE 1

MINIMAL INHIBITORY CONCENTRATION (ug/ml) of AMPHIPHILIC PEPTIDES VERSUS ENTEROBACTER CLOACAE (Panel A: Absence of NaF) (Panel B: + 50 mM NaF)		
MGN2 (Magainin II)	>500 ug/ml	60-125
PGLa	250-500	60-125
Z-44	>500	30-60

TABLE II

MINIMAL INHIBITORY CONCENTRATION (ug/ml) OF AMPHIPHILIC PEPTIDES VERSUS PSEUDOMONAS AERUGINOSA (Panel A: Absence of NaF) (Panel B: + 50 mM NaF)		
MGN 2	250-500	60-125
PGLa	>500	60-125
Z-44	30-60	6-15

Approximately 1,000 bacteria were seeded into about 200 ul of trypticase soy broth. Either magainin 2 carboxy terminal amide, PGLa, or ALSKALSK ALSKALSKALSKALSK (Z-44) were added in increasing concentrations in the absence (Panel A) or presence

-20-

(Panel B) of 50 mM NaF. As can be seen, in the absence of NaF, the MIC against *Enterobacter cloacae* exceeded 500 ug/ml for MGN2, 250 ug/ml for PGLa, and 500 ug/ml for Z-44. In the presence of 50 mM NaF, the MIC against the same organism fell to between 60 to 125 ug/ml for MGN2 and PGLa, and between 30 ug/ml and 60 ug/ml for Z-44. Similar effects were noted for *Pseudomonas aeruginosa* (TABLE II).

The effect on potentiation can be demonstrated in another type of experiment (TABLE III). If 10^7 *E. cloacae* organisms are inoculated into 1 ml of TSB in the presence of 60 ug/ml of MGN2, about 10^6 bacteria are found to be viable on plating. If 50 mM NaF is included in the incubation containing MGN2, all bacteria are killed. The effective killing power of this antibiotic at a given concentration has been potentiated over 1 million-fold by the addition of fluoride.

TABLE III

Addition	CFU	EOP (% viable)
None	10^7	100
MGN2(NH ₂) (60ug/ml)	10^6	90
MGN2 (NH ₂) (60ug/ml)		
+NaF 25 mM	0	0
NaF 25mM	10^7	100

LEGEND: Organisms were incubated in a volume of 1 ml in either trypticase soy broth alone or containing substances noted for 15 min at 37° C. An aliquot was then diluted and plated to determine number of viable organisms.

-21-

The effect of fluoride as a potentiator can be demonstrated with tumor cells. If MGN2 or Z-44 is added to minimal Eagle's medium containing 10% fetal calf serum and incubated in the presence of Vero cells (a malignant epithelial cell line) approximately 50% of the cells are rendered trypan blue permeable within 30 minute at between 250 and 500 ug/ml MGN2 or Z-44. The extent of cell damage remains relatively constant over a subsequent twenty-four hour incubation at 37° C. If NaF is added to the incubation medium containing MGN2 or Z-44, 50% killing is observed at between 125 and 250 ug/ml MGN2 or Z-44.

The peptide or protein and toxic anion, as hereinabove described, may be employed for treating a wide variety of hosts. In accordance with a preferred embodiment, a host is an animal, and such animal may be a human or non-human animal. The peptide or protein and toxic anion may be employed together in a single composition, or in separate compositions. Moreover, the toxic anion and the peptide or protein may be delivered or administered in different forms, for example, the toxic anion may be administered orally, while the peptide or protein may be administered by IV or IP.

The peptide or protein and/or toxic anion may be employed in a wide variety of pharmaceutical compositions in combination with a non-toxic pharmaceutical carrier or vehicle such as a filler, non-toxic buffer, or physiological saline solution. Such pharmaceutical compositions may be used topically or systemically and may be in any suitable form such as a liquid, solid, semi-solid, injectable solution, tablet, ointment, lotion, paste, capsule, or the like. The peptide or protein and/or toxic anion may also be used in combination with adjuvants, protease inhibitors, or compatible drugs where such a combination is seen to be desirable r advantageous

-22-

in controlling infection caused by harmful microorganisms including protozoa, viruses, and the like.

The peptide(s) or protein of the present invention may be administered to a host; in particular an animal, in an effective antibiotic and/or anti-tumor and/or anti-viral and/or anti-microbial and/or an antispermicidal amount and/or anti-parasitic amount in conjunction with a toxic anion for potentiating the activity of the peptide or protein.

As representative examples of administering the peptide or protein and toxic anion for topical or local administration, the peptide could be administered in an amount of up to about 1% weight to weight and the toxic anion delivered in an amount of about 50 mM (about 0.1%). Alternatively, the toxic anion, in the form of a salt such as sodium fluoride could be administered orally in conjunction with systemic administration of the peptide and/or protein. For example, the peptide or protein may be administered IV or IP to achieve a serum dose of 100 micrograms per milliliter (10 milligrams per kilogram) in conjunction with an oral dose of toxic anion, in particular, sodium fluoride of 10 meq. per kilogram.

The peptide or protein (in particular, the peptide), and toxic anion may be employed in the form of an oral composition for oral hygiene. Such a composition may be incorporated into a wide variety of compositions and materials used for oral hygiene purposes, which include, but are not limited to, toothpastes, mouthwashes, tooth gels, and tooth powders. Such a composition may thus be used to treat or prevent periodontal disease, to prevent or reduce plaque, and/or to prevent or treat or reduce dental caries. The peptide or protein and toxic anion may be used to inhibit, prevent, or destroy the growth of Streptococcus mutans, which is associated with dental caries and periodontal disease.

-23-

Numerous modifications and variations of the present invention are possible in light of the above teachings and, therefore, within the scope of the appended claims, the invention may be practiced otherwise than as particularly described.

-24-

WHAT IS CLAIMED IS

1. A process comprising:
administering to a host at least one
biologically active amphiphilic peptide and/or
biologically active protein, said peptide or protein being
an ion channel-forming peptide or protein; and a toxic
anion, said components being administered in an amount to
inhibit growth of a target cell in the host.
2. The process of Claim 1 wherein the peptide is a
magainin peptide.
3. The process of Claim 1 wherein the peptide is a
cecropin.
4. The process of Claim 1 wherein the peptide is a
sarcotoxin.
5. The process of Claim 1 wherein the peptide is a
XPF peptide.
6. The process of Claim 1 wherein the peptide is a
PGLa peptide.
7. The process of Claim 1 wherein the peptide is a
CPF peptide.
8. The process of Claim 1 wherein the toxic anion
is selected from the group consisting of fluoride,
bicarbonate and peroxide.
9. The process of Claim 1 wherein the anion is
fluoride.
10. The process of Claim 1 wherein the peptide and
fluoride are administered separately.
11. The process of Claim 9 wherein the fluoride is
administered topically.
12. The process of Claim 9 wherein the peptide is
administered topically.
13. The process of Claim 9 wherein the peptide and
fluoride are both administered topically.
14. The process of Claim 9 wherein the peptide and
fluoride are administered in effective antibiotic amounts.

-25-

15. A composition comprising:

(a) at least one biologically active amphiphilic peptide and/or biologically active protein, said peptide or protein being an ion channel-forming peptide or protein; and

(b) a toxic anion.

16. The composition of Claim 15 wherein said components (a) and (b) are present in an amount to inhibit growth of a target cell.

17. The composition of Claim 15 wherein the anion is fluoride.

18. The composition of Claim 17 wherein the peptide is a magainin peptide.

19. The composition of Claim 17 wherein the peptide is a cecropin.

20. The composition of Claim 17 wherein the peptide is a sarcotoxin.

21. The composition of Claim 17 wherein the peptide is a XPF peptide.

22. The composition of Claim 17 wherein the peptide is a PGLa peptide.

23. The composition of Claim 17 wherein the peptide is a CPF peptide.

24. A method of testing an unknown sample for the presence of ion channel-forming peptides, comprising:

contacting a first portion of said unknown sample with a first sample of target cells;

measuring the biological activity of said first portion against said target cells;

contacting a second portion of said unknown sample, and a given amount of fluoride ion with a second sample of target cells, said second sample of target cells containing the same type of target cells as said first sample;

-26-

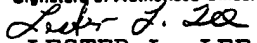
measuring the biological activity of said second portion of said unknown sample and said fluoride ion against said second sample of target cells; and

comparing the biological activity of said first portion of said unknown sample and said second portion of said unknown sample and said fluoride ion against said target cells to determine the presence of ion channel-forming peptides in said unknown sample.

25. The method of Claim 24 wherein said target cell is a bacterium.

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US89/04664

I. CLASSIFICATION F SUBJECT MATTER (if several classification symbols apply, indicate all) ⁶		
According to International Patent Classification (IPC) or to both National Classification and IPC		
IPC(4): A61K 37/02; C07K 7/08,10		
U.S. CL: 514/12,13,14; 530/324,325,326		
II. FIELDS SEARCHED		
Minimum Documentation Searched ⁷		
Classification System	Classification Symbols	
U.S.	514/12,13,14 530/324,325,326	
Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched ⁸		
III. DOCUMENTS CONSIDERED TO BE RELEVANT ⁹		
Category ¹⁰	Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹²	Relevant to Claim No. ¹³
A	US, A, 4,507,230 (TAM ET AL) 26 March 1985. See entire document.	1-25
<p>¹⁰ Special categories of cited documents:</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"&" document member of the same patent family</p>		
IV. CERTIFICATION		
Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report	
07 December 1989	22 JAN 1990	
International Searching Authority	Signature of Authorized Officer	
ISA/US	 LESTER L. LEE	